AMENDMENTS TO THE CLAIMS:

Claims 1, 10, 20, 21 and 59-69 are amended. The following is the status of the claims of the above-captioned application, as amended.

1. (Currently amended) A method for producing a variant of a parent pullulanase, wherein the parent pullulanase has more than 50% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~ 4 or SEQ ID NO:~~5~~ 6, and wherein the variant has at least one altered property as compared to the parent pullulanase, the method comprising:

a) modeling the parent pullulanase on the three-dimensional structure of SEQ ID NO:~~1~~2 depicted in Appendix 1 to produce a three-dimensional structure of the parent pullulanase;

b) identifying in the three-dimensional structure obtained in step (a) at least one structural part of the parent pullulanase, wherein an alteration in said structural part is predicted to result in an altered property;

c) modifying the nucleic acid sequence encoding the parent pullulanase to produce a nucleic acid sequence encoding a variant of the parent pullulanase having a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and

d) expressing the modified nucleic acid sequence in a host cell to produce the variant pullulanase.

2. (Previously presented) The method of claim 1, wherein the altered property is pH dependent activity, thermostability, substrate cleavage pattern, specific activity of cleavage, substrate specificity or substrate binding.

3. (Previously presented) The method of claim 2, wherein the altered property is a higher isoamylase activity as defined by an increase of at least 5% in the number of reducing ends formed in an "assay for isoamylase-like activity" using 50 mM sodium acetate, a pH of 4.5, 5.0 or 5.5, a temperature of 60°C and when incubated with a 10% w/v rabbit liver glycogen solution for a period of 10 min.

4. (Previously presented) The method of claim 1, wherein the altered property is an improved thermostability as defined by differential scanning calorimetry (DSC).

5. (Previously presented) The method of claim 1, wherein the altered property is an improved thermostability as defined by an increased half-life ($T_{½}$) of at least about 5% in a "$T_{½}$ assay for liquefaction", using a pH of 5.0 and a temperature of 95°C.

6. (Previously presented) The method of claim 1, wherein the altered property is an improved thermostability as defined by an increased residual enzyme activity of at least about 5% in an "assay for residual activity after liquefaction", using a pH of 5.0 and a temperature of 95°C.

7. (Previously presented) The method of claim 1, wherein the altered property is an improved thermostability as defined by an increased half-life ($T_{½}$) of at least about 5% in a "$T_{½}$ assay for saccharification", using a pH of 4.5 and a temperature of 70°C.

8. (Previously presented) The method of claim 1, wherein the altered property is an improved thermostability as defined by an increased residual enzyme activity of at least about 5% in an "assay for residual activity after saccharification", using a pH of 4.5 and a temperature of 63°C.

9. (Previously presented) The method according to claim 8, wherein the "assay for activity for saccharification", is carried out at a pH of 4.5 and at a temperature of 70°C.

10. (Currently amended) A method for constructing a variant of a parent pullulanase, wherein the parent pullulanase has more than 50% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~2 or SEQ ID NO:~~5~~6, and wherein the variant has at least one altered property as compared to the parent pullulanase, the method comprising:

a) modeling the parent pullulanase on the three-dimensional structure of SEQ ID NO:~~1~~2 depicted in Appendix 1 to produce a three-dimensional structure of the parent pullulanase;

b) identifying an internal or external cavity or crevice in a three-dimensional structure of the parent pullulanase;

c) substituting at least one amino acid residue in the vicinity of the cavity or crevice with another amino acid residue which increases the hydrophobic interaction and/or fills out or reduces the size of the cavity or crevice;

d) preparing the variant resulting from steps b) and c);

e) testing the thermostability of the variant; and

f) selecting a variant having increased thermostability as compared to the parent pullulanse.

Claims 11-14 (Cancelled.)

15. (Previously presented) A method according to claim 10, wherein the increased thermostability is as defined by differential scanning calorimetry.

Claims 16-19 (Cancelled.)

20. (Currently amended) A method according to claim 1, wherein the parent pullulanse has more than 60% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

21. (Currently amended) A method according to claim 20, wherein the parent pullulanase has the amino acid sequences shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

22. (Previously presented) A method for producing a pullulanase variant, the method comprising:

constructing the variant by the method according to claim 10;

transforming a microorganism with a DNA sequence encoding the variant; and

cultivating the transformed microorganism under conditions which are conducive for producing the variant.

Claims 23-57 (Cancelled.)

58. (Previously presented) The method of claim 22, wherein the method further comprises recovering the variant from the cultivation.

59. (Currently amended) A method according to claim 1, wherein the parent pullulanse has more than 70% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

60. (Currently amended) A method according to claim 1, wherein the parent pullulanse has more than 80% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

61. (Currently amended) A method according to claim 1, wherein the parent pullulanse has more than 90% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

62. (Currently amended) A method according to claim 1, wherein the parent pullulanse has more than 95% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

63. (Currently amended) A method according to claim 1, wherein the parent pullulanse has more than 99% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

64. (Currently amended) A method according to claim 10, wherein the parent pullulanse has more than 60% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

65. (Currently amended) A method according to claim 10, wherein the parent pullulanse has more than 70% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

66. (Currently amended) A method according to claim 10, wherein the parent pullulanse has more than 80% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

67. (Currently amended) A method according to claim 10, wherein the parent pullulanse has more than 90% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

68. (Currently amended) A method according to claim 10, wherein the parent pullulanse has more than 95% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

69. (Currently amended) A method according to claim 10, wherein the parent pullulanse has more than 99% homology with the amino acid sequence shown in SEQ ID NO: ~~1~~2, SEQ ID NO: ~~3~~4 or SEQ ID NO: ~~5~~6.

70. (Currently amended) The method of claim 10, wherein said method comprises repeating steps b) and c) recursively.